

CLAIMS

1 1. A mutant form of human dihydrofolate reductase which differs from
2 wild-type human dihydrofolate reductase at both amino acid 22 and amino acid 31, wherein
3 the mutant form has an amino acid with a larger volume side chain than leucine at amino acid
4 22 and an amino acid which having a smaller volume, more hydrophilic side chain than
5 phenylalanine at amino acid 31.

1 2. The mutant form of human dihydrofolate reductase according to claim
2 1, wherein the amino acid at amino acid 22 is selected from phenylalanine and tyrosine, and
3 the amino acid at amino acid 31 is selected from alanine, serine and glycine.

1 3. The mutant form of human dihydrofolate reductase according to claim
2 1, wherein the amino acid at amino acid 22 is phenylalanine and the amino acid at amino acid
3 31 is serine.

1 4. cDNA encoding a mutant form of human dihydrofolate reductase
2 which differs from wild-type human dihydrofolate reductase at both amino acid 22 and amino
3 acid 31, wherein the mutant form has an amino acid with a larger volume side chain than
4 leucine at amino acid 22 and an amino acid which having a smaller volume, more hydrophilic
5 side chain than phenylalanine at amino acid 31.

1 5. The cDNA according to claim 4, wherein the amino acid at amino acid
2 22 is selected from phenylalanine and tyrosine, and the amino acid at amino acid 31 is selected
3 from alanine, serine and glycine.

1 6. The cDNA according to claim 4, wherein the amino acid at amino acid
2 22 is phenylalanine and the amino acid at amino acid 31 is serine.

1 7. A DNA vector comprising DNA encoding a mutant form of human
2 dihydrofolate reductase which differs from wild-type human dihydrofolate reductase at both
3 amino acid 22 and amino acid 31, wherein the mutant form has an amino acid with a larger

4 volume side chain than leucine at amino acid 22 and an amino acid which having a smaller
5 volume more hydrophilic side chain than phenylalanine at amino acid 31.

1 8. A mammalian cell which produces a mutant form of human
2 dihydrofolate reductase which differs from wild-type human dihydrofolate reductase at both
3 amino acid 22 and amino acid 31, wherein the mutant form has an amino acid with a larger
4 volume side chain than leucine at amino acid 22 and an amino acid which having a smaller
5 volume more hydrophilic side chain than phenylalanine at amino acid 31 inserted into a site
6 which is not essential for replication of the vector.

1 9. The mammalian cell of claim 8, wherein the cell is a hematopoietic cell.

1 10. The mammalian cell of claim 9, wherein the cell is a peripheral blood
2 stem cell.

1 11. The mammalian cell of claim 11, wherein the amino acid at amino acid
2 22 is selected from phenylalanine and tyrosine, and the amino acid at amino acid 31 is selected
3 from alanine, serine and glycine.

1 12. A method for reducing the toxic effects of antifolate therapy on human
2 cells comprising the step of introducing into the cells an expressible mutant form of human
3 dihydrofolate reductase which differs from wild-type human dihydrofolate reductase at both
4 amino acid 22 and amino acid 31, wherein the mutant form has an amino acid with a larger
5 volume side chain than leucine at amino acid 22 and an amino acid which having a smaller
6 volume more hydrophilic side chain than phenylalanine at amino acid 31.

1 13. The method according to claim 12, wherein the amino acid at amino
2 acid 22 is selected from phenylalanine and tyrosine, and the amino acid at amino acid 31 is
3 selected from alanine, serine and glycine.

1 14. The method according to claim 12, wherein the amino acid at amino
2 acid 22 is phenylalanine and the amino acid at amino acid 31 is serine.

1 15. The method of claim 12, wherein the antifolate is methotrexate.

1 16. A method for reducing the toxic effects of antifolate therapy in a human
2 patient, comprising the steps of

3 (a) obtaining hematopoietic cells from the patient;

4 (b) transducing into the hematopoietic cells an expressible mutant form of
5 human dihydrofolate reductase which differs from wild-type human dihydrofolate reductase at
6 both amino acid 22 and amino acid 31, wherein the mutant form has an amino acid with a
7 larger volume side chain than leucine at amino acid 22 and an amino acid which having a
8 smaller volume, more hydrophilic side chain than phenylalanine at amino acid 31; and

9 (c) returning the transduced cells to the human patient.

1 17. The method according to claim 16, wherein the amino acid at amino
2 acid 22 is selected from phenylalanine and tyrosine, and the amino acid at amino acid 31 is
3 selected from alanine, serine and glycine.

1 18. The method according to claim 16, wherein the amino acid at amino
2 acid 22 is phenylalanine and the amino acid at amino acid 31 is serine.

1 19. The method of claim 16, wherein the antifolate is methotrexate.

1 20. A method for selecting among clones for clones expressing a non-
2 selectable gene, comprising the steps of:

3 (a) inserting the non-selectable gene into a DNA vector comprising DNA
4 encoding a mutant form of human dihydrofolate reductase which differs from wild-type human
5 dihydrofolate reductase at both amino acid 22 and amino acid 31, wherein the mutant form has
6 an amino acid with a larger volume side chain than leucine at amino acid 22 and an amino acid
7 which having a smaller volume more hydrophilic side chain than phenylalanine at amino acid

8 31 wherein the non-selectable gene is inserted into a site which is not essential for replication
9 of the vector;

10 (b) introducing the vector containing the non-selectable gene into cells of a
11 type in which the non-selectable gene and the mutant form of dihydrofolate reductase are
12 expressed; and

13 (c) selecting cells which are resistant to inhibition by antifolates.